Population pharmacokinetic modelling of NS2330 (tesofensine) and its major metabolite in patients with Alzheimer's disease

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What is already known about this subject

- Several studies in predominantly healthy subjects have investigated the pharmacokinetics of NS2330 and its major metabolite M1.
- However, its pharmacokinetics have not been characterized in Alzheimer's disease patients, the target population for NS2330.
- In addition, no covariates have previously been found to influence the plasma concentration-time profiles of NS2330 and/or M1.

What this study adds

- A descriptive and predictive population pharmacokinetic model for NS2330 and its metabolite was successfully developed in a population of patients with Alzheimer's disease.
- A covariate analysis elucidated sex and creatinine clearance as having an influence on the plasma concentration-time profiles of NS2330 after long-term treatment.

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Aims

To develop a population pharmacokinetic model for NS2330 and its major metabolite M1 based on data from a 14 week proof of concept study in patients with Alzheimer's disease, and to identify covariates that might influence the pharmacokinetic characteristics of the drug and/or its metabolite.

Methods

Plasma data from 320 subjects undergoing multiple oral dosing, and consisting of 1969 NS2330 and 1714 metabolite concentrations were fitted simultaneously using NONMEM.

Results

Plasma concentration-time profiles of NS2330 and M1 were best described by one-compartment models with first-order elimination for both compounds. Absorption of NS2330 was best modelled by a first-order process. Low apparent clearances together with large apparent volumes of distribution resulted in long half-lives of 234 h (NS2330) and 374 h (M1). The covariate analysis identified weight, sex, CL_{CR} , BMI and age as influencing the pharmacokinetics of NS2330 and/or M1. However, simulations performed revealed that only CL_{CR} and sex had a significant effect on the steady-state plasma concentration-time profiles. Females with a creatinine clearance of 35.6 ml min^{-1} showed a 62% increased exposure compared with males without renal impairment. The robustness and accuracy of the model were demonstrated by the successful predictivity of an external dataset.

Conclusions

A descriptive, robust and predictive model for NS2330 and its M1 metabolite was developed. Important covariates influencing pharmacokinetics were identified, which might guide the further development of NS2330 and optimize its long-term use in the treatment of Alzheimer's disease.

Introduction

Alzheimer's disease (AD) accounts for 60% to 70% of cases of progressive cognitive impairment in elderly patients [1]. The prevalence of AD doubles every 5 years after the age of 60 years from approximately 1% among 60–64 year olds to about 40% of those aged 85 years and older [2]. Accordingly, there is an urgent need for new therapeutic approaches for the treatment of AD. Currently there are only five drugs approved by the US Food & Drug Administration (FDA) for its treatment, and only a small number of candidate compounds are in the late clinical phases of development [3].

One of the promising new drugs for AD is NS2330 (8-azabicyclo [3,2,1]octane,3-(3,4-dichlorophenyl)-2-(ethoxymethyl)-8-methyl-, [1R-(2-endo,3-exo)]-, 2-hydroxy-1,2,3-propanetricarboxylate) [4]. *In vitro* and *in vivo* investigations have revealed that NS2330 inhibits the presynaptic uptake of the neurotransmitters norepinephrine, dopamine and serotonin. In addition, the cholinergic system is indirectly stimulated [4]. Moreover, there was a decrease in amyloid- β (A β) concentrations after NS2330 treatment in mice [5]. As A β is thought to be a gatekeeper protein in the pathogenesis of AD [6], decreased A β production might result in a neuroprotective effect and a slowing of disease progression. AD patients might benefit initially from the increase in the different neurotransmitter concentrations, and on a long-term basis from the decreased disease progression during NS2330 treatment. Several preclinical studies have shown an enhancement in cognitive function, short- and long-term memory and attention in animals (unpublished observations). In a phase IIa trial performed in mild AD patients, NS2330 demonstrated a significant improvement in cognitive function [4].

Several clinical studies have been performed to investigate the absorption, distribution, metabolism and excretion of NS2330, predominantly in healthy subjects (unpublished observations). Dose-proportionality was demonstrated after both single and multiple dose administration over the ranges 1–10 mg and 0.25–0.5 mg four times daily, respectively. The maximum plasma concentration was reached after 6–8 h. The absolute bioavailability of NS2330 was estimated to be >90% by comparing the results of separate i.v. and p.o. studies. After i.v. administration NS2330 displayed a high volume of distribution of about 600 l and a low oral clearance of 30–40 ml min⁻¹. No clinically relevant difference in the pharmacokinetics of NS2330 between fed and fasted subjects has been found. NS2330 is mainly metabolized by cytochrome P450 3A4 (CYP3A4) into its desalkyl metabolite M1. Parent compound and metabolite revealed markedly long apparent half-lives in

humans of ~200 h [7] and ~400 h, respectively. M1 is the only metabolite detectable in human plasma and shows the same pharmacological profile as NS2330. *In vivo* investigations in mice have revealed a five-fold lower potency of the metabolite compared with the parent compound [8].

Only limited information on the pharmacokinetics of NS2330 in the target population has been obtained. Additionally, no steady-state (reached in 6–12 weeks for the parent compound and the metabolite) observations have yet been made. As the drug is intended to be administered as a long-term treatment in AD patients, the knowledge of the pharmacokinetics of NS2330 and its metabolite M1 needs to be expanded. Furthermore, because AD patients often suffer from multimorbidities and often experience polypharmacy, the identification by population analysis, of covariates influencing the pharmacokinetics of NS2330 should help to guide the selection of dose regimens.

Therefore, the primary aim of this work was to develop a population pharmacokinetic model for NS2330, administered as long-term treatment, and its active metabolite M1, based on data obtained from the proof of concept study (unpublished observations) in AD patients. A secondary aim was to identify patient characteristics that might influence the pharmacokinetics of NS2330 and/or its metabolite M1. Simulations were then performed to evaluate the influence of these covariates on pharmacokinetics. To assess the robustness of the final model, its predictive performance was evaluated using an independent data set.

Methods

Study design and population

A double-blind, randomized, placebo-controlled proof of concept study (clinical phase II) was conducted in patients with mild to moderate dementia of Alzheimer's type at 63 sites in the United States and 10 sites in Canada. The objective of this parallel group study was to characterize the pharmacokinetics of NS2330 after 14 weeks treatment with 0.25, 0.5 and 1 mg between March 2003 and March 2005. The study was performed in accordance with the principles laid down in the Declaration of Helsinki (1996 Version) [9], the ICH Harmonized Tripartite Guideline for Good Clinical Practice (GCP) [10] and appropriate regulatory requirements. Written informed consent was obtained from each patient before the study began.

The main inclusion criteria were (a) 40–85 year old males or females and (b) a mild to moderate AD (Alzheimer's Disease Assessment Scale measuring cognitive features >12 [11], and Mini-Mental-State-Examination

10–24 [12]). Co-administration of drugs approved for the treatment of AD (donepezil, galantamine, rivastigmine, tacrine) was not permitted.

NS2330 (0.25, 0.5, 1 mg) or placebo were supplied as oral tablets, all identical in appearance, such that the four treatment groups were indistinguishable. Patients were asked to take one tablet every day preferably in the morning, for 14 weeks.

Data collection

Blood samples for the determination of NS2330 and M1 in plasma were drawn at the following time points: before and 3–6 h after administration of the first dose, at the beginning of the clinic visits and 3–6 h later after 4, 9 and 14 weeks of treatment, and at any time during the clinic visit during week 20 (6 weeks after the last dose). Accordingly, nine plasma samples were planned to be taken from each patient. Additionally, a more intensive sampling protocol was used in a small group of 14 patients at week 14 (predose, 2, 4, 6, 8, and 12 h after administration). In most cases the samples taken at the beginning of the visits were trough values. However, some patients changed their normal dosing time, which meant that the samples taken at the beginning of the visit were not trough samples.

Demographic data were documented at the baseline visit, laboratory data were obtained additionally at weeks 4, 14 and 20, and body weight was recorded additionally at weeks 2, 5, 6, 7, 8 and 9.

Drug and metabolite analysis

NS2330 and M1 concentrations in plasma were determined by high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) using deuterated NS2330 and deuterated M1 as internal standards. The method was validated according to the current FDA guidance on bioanalytical method validation [13].

The HPLC-MS/MS system consisted of Shimadzu LC 10ADvp HPLC pumps (Shimadzu, Duisburg, Germany), a CTC HTS PAL autosampler (Axel Semrau, Sprockhoevel, Germany) and a Waters (Micromass) Quattro LC mass spectrometer equipped with a Z-Spray ion source (Waters, Eschborn, Germany). The system was operated using the Masslynx software version 3.5. HPLC was performed using a Phenomenex Luna C18(2) column (dimension 30 × 2.0 mm), with gradient elution. The mobile phase consisted of 0.1% formic acid (solvent A) and methanol (solvent B). The analytical run time was 7.0 min at a flow rate of 0.2 ml min⁻¹ (entire eluent was directed to the mass spectrometer). The retention time for NS2330 was 4.7 min, that for M1 4.8 min. The

respective internal standards were co-eluting with unlabelled drug and its metabolite. Samples were cleaned up by automated solid phase extraction in 96-well plates using an Isolute HXC 100 mg extraction plate. The calibration function for both analytes was linear from 0.1 to 50 ng ml⁻¹ using a plasma volume of 200 µl. Assay performance was assessed by back-calculation of calibration solutions of eight different concentrations, and analysis of quality control (QC) samples at five different concentrations, including a quality control sample at the lower limit of quantification, as well as a dilution quality control sample. Values of inaccuracy and imprecision were <±6% (relative error) and <11% (coefficient of variation) (*n* = at least 10), respectively.

Data analysis

The population PK analyses were performed using NONMEM software, version V [14]. The First Order Conditional Estimation method with interaction and the ADVAN5 subroutine were used. Population parameters and their standard errors expressed as percentages were estimated. Goodness-of-fit was assessed using the objective function value (OFV) [15] and other diagnostic methods [16]. If models were classified as nested, one model was declared superior to the other if the OFV was decreased by 3.84 (*P* < 0.05 with 1 degree of freedom) [14].

Individual terminal half-lives were calculated from the individual pharmacokinetic parameter estimates provided from a maximum *a posteriori* probability Bayesian fitting method according to the following equations:

$$t_{1/2, \text{NS2330}} = \frac{\ln 2 \times V_2 / F}{\text{CL}_{\text{non-met}} + \text{CL}_{\text{met}}} \quad (1)$$

$$t_{1/2, \text{M1}} = \frac{\ln 2 \times V_3 / F}{\text{CL}_{\text{M1}}} \quad (2)$$

Modelling strategy

The development of a combined population PK model was started using a primary dataset when approximately two thirds (254 out of 320) of all the patients in the treatment arm had completed the study. After database lock the remaining one third of patients were added to the primary dataset for model evaluation.

The population PK modelling of the combined model of NS2330 and M1 was performed in a sequential manner. First, a final base model for each compound was built in parallel. For the modelling of M1, the dosing information for NS2330 was used. NONMEM control files were parameterized with respect to apparent volumes of distribution, distribution and elimination

clearances, and absorption rate constants. One-, two- and three- compartment models were developed for NS2330 and M1 individually. A series of pharmacostatistical models was systematically evaluated in order to identify the one that best described the data. Initially, interindividual variability (IIV) was modelled using exponential random effects models, and residual variability using a combined (additive + proportional) error model separately for NS2330 and M1. Other models were investigated based on the results obtained and on graphical analysis. Second, a combined model was built, based on the information gained from the individual analysis. Initially, the structural models obtained by the individual analyses were used, using the data from the formation of M1 from NS2330 to link the two models. Different formation mechanisms (first-order, zero-order, saturable) were evaluated. Structural modifications were also investigated (addition or removal of compartments). IIV and residual variability were reassessed in the combined model.

A covariate analysis was performed using the final base model for NS2330 and M1. A general approach was applied with the following steps: (a) candidate selection: prespecified physiologically plausible covariates and those identified by a GAM procedure [17] were selected, (b) univariate implementation into the model, (c) forward inclusion and (d) backward elimination. For (b), covariates preselected were incorporated univariately into the final base model. Continuous covariates were added to the structural model using a linear relationship or a hockey stick function, and categorical covariates were implemented as a step function [17]. Covariates producing a decrease of >3.84 points in the OFV were used for the forward inclusion step. If highly correlated covariates were identified, e.g. body weight and BMI, only the most significant covariate was used for further investigations. In the forward inclusion step (c), the covariates with the strongest influence obtained by the univariate implementation were retained in the population model, whereas the other covariates were incorporated into the model sequentially according to their ranking order of statistical significance. If the additional covariate caused a significant drop in OFV (>-3.84), the covariate was retained in the model, otherwise the next covariate in the ranking order was incorporated. This forward inclusion procedure was continued until all covariates had been tested to obtain the full covariate model. For the backward elimination step (d), a stricter significance level of $P < 0.001$ (d.f. = 1) was applied. During the backward elimination each covariate was removed separately from the model. If the deletion of one or more covariates did not result in a significant

increase of 10.83 points in the OFV, the covariate with the smallest nonsignificant increase was removed from the model. This backward elimination process was stopped when the removal of each remaining covariate resulted in a significant deterioration of the model fit ($\Delta\text{OFV} > +10.83$), resulting in the 'final covariate model'. If this model contained a highly correlated covariate as described above, it was substituted for another significant covariate, and the model was reassessed. The superior covariate was retained in the final model. Discrete levels of categorical covariates were only formally tested if they were represented in at least 10% of the patient population (time invariant) or in at least 10% of the observations (time varying continuous covariates). Covariates which showed a clear trend during the graphical analysis, but were present in less than 10% of the population/observations, were additionally explored in the final covariate model for information purposes.

To assess the influence of covariates on the plasma concentration-time profile of NS2330 and M1, simulations were performed. For these a dosing regimen of 1 mg NS2330 administered orally once daily for 4000 h (166 days) was assumed. Model and parameter estimates from the final covariate model obtained with the final evaluation dataset were used for simulations. These were performed using Berkeley Madonna software (Berkeley Madonna, Version 8.0.1, 2000), and graphs were generated using SigmaPlot (SPSS, Version 8.0, 2002).

Model evaluation

Internal and external evaluations were performed. In the former, the remaining one third of the data were added to the primary dataset resulting in a final dataset. The final base and covariate models containing the primary dataset were re-evaluated using the final evaluation dataset. Parameter estimates were compared numerically, and a visual comparison of typical plasma concentration-time profiles was carried out by simulation. The final base model containing the final evaluation dataset was used to reassess the relationship between the pharmacokinetic parameters and the available covariates, in order to determine whether new relations might have arisen through adding the remaining patients. Any new relations were further investigated. A backward elimination was performed on the final model to evaluate whether these covariates remained significant.

For external evaluation a randomized, double-blind, placebo-controlled, five parallel group study of NS2330 (at doses of 0.125 mg, 0.25 mg, 0.5 mg and 1.0 mg) administered orally once daily over 14 weeks in 202 levodopa-treated Parkinson patients with motor fluctua-

Table 1

Continuous covariates in the AD population studied

Covariate		Median	Mean	Range	5th–95th percentile
Age (years)	AGE	76	75	45–91	59–86
Weight (kg)	WT	68.7	71.1	37.2–136.1	47.6–102.5
Height (cm)	HT	163	163	132–201	147–183
Body mass index (kg m ⁻²)	BMI	25.6	26.4	14.5–51.3	19.8–36.3
Serum creatinine (mg dl ⁻¹)	S _{CR}	0.9	0.9	0.2–1.8	0.6–1.3
Creatinine clearance* (ml min ⁻¹)	CL _{CR}	63.1	69.0	24.5–358.1	35.6–117.2
Alanine transferase (U l ⁻¹)	ALT	16	18	4–106	9–32
Aspartate transaminase (U l ⁻¹)	AST	20	21	10–135	13–30
Alkaline phosphatase (U l ⁻¹)	AP	79	82	24–219	49–124
Bilirubin (mg dl ⁻¹)	BIL	0.4	0.4	0.1–1.8	0.2–0.8

*Calculated according to Cockcroft & Gault [24].

tions between April 2003 and February 2005, was used. Plasma concentrations of NS2330 and M1 were simulated based on individual dosing history and demographic characteristics. Simulations were performed using the final combined pharmacokinetic model and by setting the number of evaluations to zero. Thus, no iteration was performed, and the population and individual predictions were estimated based on the parameter estimates obtained from the final model. The quality of the evaluation was assessed by graphical presentation of the observed concentration *vs.* the population and individual predictions. In addition, the distribution of the individual estimates η of interindividual variability was investigated graphically, to evaluate whether they were normally distributed.

Results

The final evaluation dataset was obtained from 320 patients and included 1969 NS2330 and 1714 M1 plasma concentrations above the lower limit of quantification. Values ranged from 0.101 to 105 ng ml⁻¹ for NS2330 and from 0.118 to 47.3 ng ml⁻¹ for M1. Owing to their physiological implausibility, 36 NS2330 and 33 M1 concentrations (1.8% of all observations) were removed *a priori* from the final evaluation dataset.

The characteristics of the population are summarized in Tables 1 and 2, and reflect the measurements taken at the start of treatment. No unexpected correlation between covariates was observed.

Plasma concentration-time profiles were best described by one-compartment models with first-order elimination processes for both compounds. Elimination

Table 2

Categorical covariates in the AD population studied

Covariate			<i>n</i>	%
Sex	SEX	Male	119	37.2
		Female	201	62.8
Ethnic origin	RAC	Caucasian	299	93.4
		African (Black)	17	5.3
		Asian	4	1.3
Smoking status	SMOK	Never smoker	170	53.1
		Ex-smoker	118	36.9
		Current smoker	32	10.0
Alcohol status	ASTA	No alcohol	181	56.6
		Average consumption	139	43.4
		Excessive consumption	0	0

of NS2330 from the central compartment was divided into an apparent nonmetabolic clearance ($CL_{\text{non-met}}/F$), which accounted for all elimination pathways except the formation of M1 from NS2330, and an apparent metabolic clearance (CL_{met}/F) that accounted for the formation of M1 from NS2330. Absorption of NS2330 was best modelled as a first-order absorption process (k_a). Different options were explored to overcome identifiable issues occurring in the assessment of parent compound and metabolite pharmacokinetics. Setting the typical volume of distribution of M1 ($V3/F$) to 0.768-fold of that of NS2330 ($V2/F$) best described the data. This value was based on prior data from mice [8] and from data obtained from allometric scaling studies (data not

Figure 1

Schematic representation of the pharmacokinetic model

shown). A schematic illustration of the pharmacokinetic model is shown in Figure 1.

The apparent volume of distribution was 653 l for NS2330 and 501.7 l for M1.

Interindividual variability was added to nonmetabolic clearance ($\omega\text{CL}_{\text{non-met}}/F$), metabolic clearance ($\omega\text{CL}_{\text{met}}/F$), the central volume of distribution of NS2330 ($\omega\text{V2}/F$) and M1 ($\omega\text{V3}/F$), and the absorption rate constant (ωk_a). Owing to very sparse sampling in 96% of patients (one sample after every 35th oral dosing), and to 4% of patients with rich sampling, but only after the last dose, interoccasion variability could not be incorporated into the model. The correlation between oral $\text{CL}_{\text{non-met}}/F$ and $\text{V2}/F$ was added to the model. Residual variability was modelled with a combined residual error model for NS2330 and a proportional residual error model for M1. Parameter estimates using the final evaluation dataset are shown in Table 3.

The covariates investigated with respect to the final base model are listed in Tables 1 and 2, and those tested during univariate implementation in Table 4. The analysis after forward inclusion and backward elimination identified weight as a statistically significant covariate on CL_{met}/F and $\text{V2}/F$. For patients with a creatinine clearance (CL_{CR}) lower than 62.5 ml min⁻¹, an influence of CL_{CR} on $\text{CL}_{\text{non-met}}/F$ was apparent. In addition, a sex difference was observed for $\text{CL}_{\text{non-met}}/F$. Body mass index and age were found to have a statistically significant influence on the distribution volume of the metabolite ($\text{V3}/F$). During model development, the highly correlated covariates weight and BMI were exchanged,

but both were retained based on statistical significance. The mathematical expressions for the relations explored are listed in Table 3. Based on these relations and on the 5th and 95th percentile covariate values of the dataset, the typical $\text{CL}_{\text{non-met}}/F$ for males will decrease from 1.52 l h⁻¹ to 1.04 l h⁻¹ and for females from 1.16 l h⁻¹ to 0.795 l h⁻¹ as creatinine clearance decreases from ≥ 62.5 to 35.6 ml min⁻¹. The typical CL_{met}/F will decrease from 0.42 to 0.352 l h⁻¹ and the typical $\text{V2}/F$ will increase from 509 l to 890 l as weight increases from 47.7 kg to 103 kg. For a typical patient with an age of 76 years (median), the typical $\text{V3}/F$ will increase from 440 to 598 l as BMI increases from 19.8 to 35.3 kg m⁻². For a typical patient with a BMI of 25.5 kg m⁻² (median), $\text{V3}/F$ will increase from 386 to 568 l as age increases from 60 to 86 years.

The low relative clearances of NS2330 and M1 in combination with the large apparent volumes of distribution resulted in half-lives of 234 h (SD 89 h) for NS2330 and of 374 h (SD 98 h) for M1.

The presence of CYP3A4 inhibitors did not fulfil the criteria for formal testing during the covariate analysis, as only less than 6% of the NS2330 plasma samples were taken in patients undergoing treatment with these drugs. Nevertheless, CYP3A4 inhibitors showed a clear trend for an effect on pharmacokinetics during graphical analysis, and consequently their influence was examined on the final covariate model. The presence of CYP3A4 inhibitors had a statistically significant influence on $\text{CL}_{\text{non-met}}/F$ and CL_{met}/F , and the apparent total clearance of NS2330 ($= \text{CL}_{\text{met}}/F + \text{CL}_{\text{non-met}}/F$) was found to be decreased by ~40% and 20% using the primary and the final evaluation dataset, respectively.

Parameter estimates from the final covariate model using the final evaluation dataset are shown in Table 3. Interindividual variability was moderate to high (70.2% in $\text{CL}_{\text{non-met}}/F$, 23.6% in CL_{met}/F , 30.5% in $\text{V2}/F$, 33.2% in $\text{V3}/F$ and 95.6% in k_a). Compared with the base model variability was decreased by 15.5% (−5.6% points) for $\text{V2}/F$, 8.7% (−6.7% points) for $\text{CL}_{\text{non-met}}/F$, 7.5% (−2.7% points) for $\text{V3}/F$, and 2.9% (−0.7% points) for CL_{met}/F . The proportional components of the residual variability were 19.4% and 19.9% for NS2330 and M1, respectively. The additive residual variability for NS2330 was ± 0.146 ng ml⁻¹.

In the final model all parameters were estimated with good precision (relative standard errors ranging from 2.2 to 45.9%, Table 3). The goodness-of-fit plots of NS2330 and M1 are shown in Figure 2. All observed vs. predicted concentrations were in general spread randomly around the line of identity, indicating that the data were well described by the model.

Table 3

Parameter estimates in the AD population studied

Parameter	Base model		Final covariate model	
	Value	RSE (%)	Value	RSE (%)
<i>a) Fixed effects</i>				
k_a (h^{-1})	0.377	8.8	0.385	8.8
$V2/F$ (l)	653	2.3	649	2.2
$CL_{\text{non-met-male}}/F$ (l h^{-1})	1.14	3.5	1.52	5.0
$CL_{\text{non-met-female}}/F$ (l h^{-1})	1.14	3.5	1.16	5.8
CL_{met}/F (l h^{-1})	0.396	3.6	0.395	3.6
$V3/F$ (l)	501.7	—	498.6	—
CL_{M1}/F (l h^{-1})	0.936	3.7	0.925	3.6

Parameter	Base model		Final covariate model	
	Value	RSE (%)	Value	RSE (%)
<i>b) Covariate influence</i>				
$V2/F_{\text{WT}}$ (%) ¹	n.a.	—	1.06	14.9
$CL_{\text{non-met}}/F_{\text{CLCR}}$ (%) ²	n.a.	—	1.17	20.9
$CL_{\text{met}}/F_{\text{WT}}$ (%) ³	n.a.	—	0.31	30.6
$V3/F_{\text{BMI}}$ (%) ⁴	n.a.	—	2.05	33.2
$V3/F_{\text{AGE}}$ (%) ⁴	n.a.	—	1.41	18.7

$$^1 V2/F = TVV2/F_{\text{individual}} \times [1 + V2/F_{\text{WT}} \times (WT - 68.04)] \times \text{EXP}(\omega V2/F);$$

$$^2 CL_{\text{CR}} < 62.5 \text{ ml min}^{-1}: CL_{\text{non-met}}/F_{\text{individual}} = CL_{\text{non-met-sex}}/F \times [1 + CL_{\text{non-met}}/F_{\text{CLCR}} \times (CL_{\text{CR}} - 62.5)] \times \text{EXP}(\omega CL_{\text{non-met}}/F)$$

$$CL_{\text{CR}} \geq 62.5 \text{ ml min}^{-1}: CL_{\text{non-met}}/F_{\text{individual}} = CL_{\text{non-met-sex}}/F \times \text{EXP}(\omega CL_{\text{non-met}}/F);$$

$$^3 CL_{\text{met}}/F_{\text{individual}} = CL_{\text{met}}/F \times [1 - CL_{\text{met}}/F_{\text{WT}} \times (WT - 68.04) \times \text{EXP}(\omega CL_{\text{met}}/F);$$

$$^4 V3/F_{\text{individual}} = V3/F \times [1 + V3/F_{\text{BMI}} \times (BMI - 25.5)] \times [1 + V3_{\text{AGE}} \times (AGE - 76)] \times \text{EXP}(\omega V2/F).$$

Parameter	Base model		Final covariate model	
	Value	RSE (%)	Value	RSE (%)
<i>c) Random effects</i>				
$\omega CL_{\text{non-met}}/F$ (CV%)	76.9	14.5*	70.2	16.1*
$\omega CL_{\text{met}}/F$ (CV%)	24.3	12.4*	23.6	13.2*
$\omega V2/F$ (CV%)	36.1	15.2*	30.5	13.8*
$\omega V3/F$ (CV%)	35.9	11.2*	33.2	12.7*
ωk_a (CV%)	96.6	17.9*	95.6	18.0*
Correlation	0.66	17.8 [§]	0.585	20.6 [§]
$CL_{\text{non-met}}/F_{V2/F}$				
prop. errNS2330 (%)	19.4	11.6*	19.4	11.6*
prop. errM1 (%)	20.0	11.5*	19.9	11.6*
add. errNS2330 (ng ml^{-1})	± 0.142	47.1*	± 0.146	45.9*

*RSE is given on the variance scale; [§]RSE of the covariance estimate.

Simulations assessing the influence of weight (Figure 3a), BMI and age (data not shown) were found to have a negligible influence on the plasma concentration-time profiles at steady-state. Exposure of

NS2330 at steady-state was 23.2% higher in females than males (Figure 3b). M1 concentrations displayed a comparable pattern. Overall, the ratio between metabolite and NS2330 steady-state concentration was approxi-

Table 4

Covariates investigated during univariate implementation

GAM		Additional covariates tested
k_a	S_{CR}	
$CL_{non-met}$	SEX, BMI, CL_{CR} , AP	CYP3A4 inhibitors*, CYP3A4 inducers*
CL_{met}	WT, ASTA	CL_{CR} , SEX, CYP3A4 inhibitors*, CYP3A4 inducers*
V2	SEX, WT, CL_{CR} , AP, BIL	BMI, BSA
V3	SEX, BMI, AGE, ASTA	WT, BSA, CL_{CR}

Abbreviations see Tables 1 and 2; *exploratively investigated, less than 10% of the observations under co-administration.

mately 1 : 3 (Figure 3b). The steady-state concentrations of NS2330 and M1 increased significantly as a result of impaired renal function (+33%). The combined effects of sex and renal function are illustrated in Figure 3c. The simulations revealed that exposure to NS2330 in a renally impaired female (CL_{CR} 35.6 ml min⁻¹) was increased by 62% compared with males with normal renal function.

The application of the final base and final covariate model developed with the primary dataset and the additional data was used to evaluate the model internally. These additional data were well described by the models, and all parameter estimates were comparable (data not shown). Simulation performed using the parameter estimates from the primary and the final evaluation dataset indicated similar plasma concentration-time profiles (data not shown). No additional covariate relations were observed and no previously detected covariate relations were found to be superfluous to the additional data.

The predicted concentrations vs. the observed concentrations from the external evaluation are shown in Figure 4. All values for the parent compound and its metabolite were evenly distributed around the line of identity, indicating that the measurements were predicted accurately by the model. Individual estimates, η , of the interindividual variability were normally distributed around zero, indicating that the estimated population parameters revealed no bias (data not shown).

Discussion

A population pharmacokinetic model for the new compound NS2330 and its major metabolite was successfully

developed in patients with Alzheimer's disease. The apparent volumes of distribution of NS2330 and M1 were found to be large, exceeding the total volume of body water, which suggests extensive distribution of both compounds into the tissues. Normalized to bodyweight, the relative volume of distribution for NS2330 was estimated to be 8.6 l kg⁻¹. Assuming F to be close to 1, this value is similar to those found in animals (after intravenous administration, the volumes of distribution of NS2330 in rats, mice, minipigs and cynomolgus monkeys were 18 l kg⁻¹, 17.7 l kg⁻¹, 8–14 l kg⁻¹ and 9.2 l kg⁻¹, respectively [8]). The apparent total clearances of NS2330 and M1 were very low. When normalized to bodyweight, NS2330 clearance was ~0.03 l h⁻¹ kg⁻¹. Other species have significantly higher clearances after intravenous administration. For example, in mice NS2330 clearance was 5.3 l h⁻¹ kg⁻¹ [8]. This difference might be caused by the low affinity of the drug for human CYP3A4 in comparison with the corresponding enzyme in the other species investigated. Species differences in other elimination pathways, or in enterohepatic circulation may also contribute to this discrepancy.

The covariate analysis identified weight, sex, CL_{CR} , BMI and age as having an influence on the pharmacokinetics of NS2330 and/or M1. The simulations showed that only CL_{CR} and sex had a significant influence on the steady-state plasma concentration-time profiles. Creatinine clearance was found to have a significant effect on the apparent nonmetabolic clearance of NS2330 in patients with a creatinine clearance of less than 62 ml min⁻¹. The apparent total clearance of NS2330 ($CL_{met}/F + CL_{non-met}/F$) was decreased by ~12 to ~25% in moderately renally impaired AD patients (CL_{CR} ; 50 ml/min to 25 ml/min) compared with those with normal renal function. These data are in close agreement findings showing that the contribution of the renal clearance to the total clearance of NS2330 is 15–20% (unpublished data).

Comparison of the relative total clearance of NS2330 between males (1.92 l h⁻¹) and females (1.56 l h⁻¹) shows a 18.7% reduction in females. In the literature several examples of sex-dependent differences in clearance have been reported where reduced as well as increased clearances in females were observed [18–23]. As the influence was found on the nonmetabolic clearance of NS2330, which comprises any elimination pathway of NS2330 except the formation into M1, different hypotheses can be generated. First, the metabolism into an additional metabolite (except M1) could be decreased in females. This hypothesis could not be investigated, as no data about sex differences in the formation of further metabolites

Figure 2

Goodness-of-fit plot based on the final model. Population predictions (1st and 3rd row) and individual predictions (2nd and 4th row) vs. observed NS2330 (first two panels) and M1 (last two panels). Plasma concentrations are shown using linear (left) and logarithmic (right) scales

was generated. Second, the renal elimination could be reduced in females. As described above NS2330 is partly eliminated via the kidney and it is known that the renal function is slightly reduced in females [24]. However, as creatinine clearance was found to have an influence on this nonmetabolic clearance as well, the sex effect seems to represent an additional effect.

Third, a reduced biliary excretion of NS2330 in females might explain the difference in the nonmetabolic clearance. There are several lines of evidence supporting this hypothesis. In rats it was shown that the biliary excretion of NS2330 was a significant part of the elimination pathway (unpublished observations). In addition, it is assumed that NS2330 might undergo

Figure 3

Influence of selected covariates on the plasma concentration-time profiles of NS2330 after oral administration of 1 mg NS2330 once daily for 166 days (4000 hours). (A) Typical profiles of a male subject with median weight as well as of subjects with a weight according to the 5th and 95th percentile of the weight distribution of the study population. (B) Typical profiles of a male and a female individual. (C) Typical profiles of a male with normal renal function, a female subject with normal renal function and a female subject with impaired renal function

enterohepatic circulation in humans which suggests biliary excretion in humans as well [25]. In the literature sex differences are reported due to different expression patterns of membrane transporters involved in biliary excretion [26]. The most well known transporter responsible for biliary excretion of drugs is P-glycoprotein (P-gp). In healthy livers, males expressed approximately 2.4-fold higher P-gp concen-

trations compared with females [27], indicating a higher biliary clearance of P-gp substrates in males.

The influence of comedication was investigated using the final model. It was found that CYP3A4 inhibitors had a statistically significant influence on the clearance of NS2330 (decreasing it by 20% to 40%, dependent on the dataset used). As only less than 6% of NS2330 plasma concentrations were obtained during treatment

Figure 4

Goodness-of-fit plot from the external validation. Population predictions (1st and 3rd row) and individual predictions (2nd and 4th row) vs. observed NS2330 (first two panels) and M1 (last two panels). Plasma concentrations are shown using a linear (left) and logarithmic (right) scale

with CYP3A4 inhibitors, this issue needs further investigation.

The volume of distribution of NS2330 was found to be influenced by body weight (~1% change per 1 kg difference from the median weight) and that of M1 was influenced by the BMI (~2% change per 1 kg m⁻² from the median value). During model development these two highly correlated covariates were exchanged, but the findings were unchanged. It is a well known that

the volumes of distribution of lipophilic drugs are correlated with body weight [28]. Extensive distribution of NS2330 and M1 into tissues was observed, which is in agreement with previous findings [8]. The simulations showed that the influences of body weight and BMI on the steady-state profiles of NS2330 and M1 were negligible. The time necessary to reach steady-state in obese patients was prolonged. To achieve this earlier in such patients, a loading dose might be appropriate.

Weight was also found to have an influence on the metabolic clearance of NS2330, although its effect on the total clearance was low (a 4% decrease in a 102 kg patient compared with a 37 kg patient). In general, the size of the liver increases proportionally with the body weight, and consequently the metabolic clearance is expected to increase [29]. However, there are examples of drugs whose volume of distribution is increased and clearance slightly decreased in obese patients [30]. One reason for this finding might be an increased distribution into other tissues at higher body weights, thus increasing volume and lowering clearance, despite the higher liver weight and increased metabolic activity. However, simulations showed that the influence of body weight on the steady-state profile of NS2330 was negligible.

The covariate analysis revealed physiologically plausible covariate effects, partly explaining the variability observed in the pharmacokinetic parameters. High interindividual variability in the nonmetabolic clearance of NS2330 still remained even after the incorporation of the sex and CL_{CR} as covariates (without considering the influence of CYP3A4 inhibitors). This indicates that there might be undiscovered covariates additionally influencing the nonmetabolic elimination of NS2330.

The covariates sex and creatinine clearance had a separate influence on the steady-state plasma concentration-time profile. Additional simulations, where the combination of both covariates in individuals, were examined resulted in a 62% increased exposure to the drug in a renally impaired female compared with a male with normal renal function. Assuming the presence of additional covariates influencing the plasma concentration-time profiles at steady-state, e.g. CYP3A4 inhibiting drugs, a larger increase in exposure might to be expected.

In summary, a descriptive, robust and predictive population PK model for the new compound NS2330 and its major metabolite in long-term treatment of AD was successfully developed. The analysis identified important covariates determining the pharmacokinetics of NS2330 in the AD population which might help to guide its further development and to optimize its clinical use.

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References

- Cummings JL, Cole G. Alzheimer disease. JAMA 2002; 287: 2335–8.
- Von Strauss E, Viitanen M, De Ronchi D, Winblad B, Fratiglioni L. Aging and the occurrence of dementia: Findings from a population-based cohort with a large sample of nonagenarians. Arch Neurol 1999; 56: 587–92.
- Benson A. Alzheimer's disease: a tangled issue. Drug Discov Today 2005; 10: 749–51.
- Thatté U. NS-2330 neurosearch. Curr Opin Invest Drugs 2001; 2: 1592–4.
- Bornemann KD, Rosenbrock H, Sams-Dodd F. Amyloid β -lowering activity of the neurotransmitter reuptake inhibitor NS 2330 *in vitro* and in an APP transgenic mouse model. 2006, submitted.
- Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 2002; 297: 353–6.
- Bara-Jimenez W, Dimitrova T, Sherzai A, Favit A, Mouradian MM, Chase TN. Effect of monoamine reuptake inhibitor NS 2330 in advanced Parkinson's disease. Mov Disord 2004; 19: 1183–6.
- Lehr T, Staab A, Tillmann C, Trommeshauser T, Schaefer HG, Kloft C. The PK/PD modeling approach for the assessment of the potency of active metabolites *in vivo*. Application to the novel CNS active drug NS2330. 2006, submitted.
- Recommendations guiding physicians in biomedical research involving human subjects. JAMA 1997; 277: 925–6.
- Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95). Step 5, Consolidated Guideline 1.5.96 including post Step 4 errata. 1996.
- Rosen WG, Mohs RC, Davis KL. A new rating scale for Alzheimer's disease. Am J Psychiatry 1984; 141: 1356–64.
- Folstein MF, Folstein SE, McHugh PR. 'Mini mental state'. A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975; 12: 189–98.
- FDA. Guidance for Industry: Bioanalytical Method Validation, 2001.
- Beal SL, Sheiner LB. NONMEM Users Guides. San Francisco, University of California, 1998.
- Akaike H. A new look at the statistical model identification. IEEE Trans Automatic Control 1974; 19: 716–23.
- Jonsson EN, Karlsson MO. Xpose – An S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM. Comput Meth Programs Biomed 1999; 58: 51–64.
- Mandema JW, Verotta D, Sheiner LB. Building population pharmacokinetic-pharmacodynamic models. I. Models for covariate effects. J Pharmacokinet Biopharm 1992; 20: 511–28.
- Beierle I, Meibohm B, Derendorf H. Gender differences in pharmacokinetics and pharmacodynamics. Int J Clin Pharmacol Ther 1999; 37: 529–47.
- Gandhi M, Greenblatt RM, Aweeka F, Blaschke TF. Sex differences in pharmacokinetics and pharmacodynamics. Annu Rev Pharmacol Toxicol 2004; 44: 499–523.
- Harris RZ, Benet LZ, Schwartz JB. Gender effects in pharmacokinetics and pharmacodynamics. Drugs 1995; 50: 222–39.

- 21 Meibohm B, Beierle I, Derendorf H. How important are gender differences in pharmacokinetics? *Clin Pharmacokinet* 2002; 41: 329–42.
- 22 Schwartz JB. The influence of sex on pharmacokinetics. *Clin Pharmacokinet* 2003; 42: 107–21.
- 23 Tanaka E. Gender-related differences in pharmacokinetics and their clinical significance. *J Clin Pharm Ther* 1999; 24: 339–46.
- 24 Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; 16: 31–41.
- 25 Roberts MS, Magnusson BM, Burczynski FJ, Weiss M. Enterohepatic circulation: Physiological, pharmacokinetic and clinical implications. *Clin Pharmacokinet* 2002; 41: 751–90.
- 26 Morris ME, Lee HJ, Predko LM. Gender differences in the membrane transport of endogenous and exogenous compounds. *Pharmacol Rev* 2003; 55: 229–40.
- 27 Schuetz EG, Furuya KN, Schuetz JD. Interindividual variation in expression of P-glycoprotein in normal human liver and secondary hepatic neoplasms. *J Pharmacol Exp Ther* 1995; 275: 1011–8.
- 28 Boxenbaum H. Interspecies scaling, allometry, physiological time, and the ground plan of pharmacokinetics. *J Pharmacokinet Biopharm* 1982; 10: 201–27.
- 29 Boxenbaum H. Interspecies variation in liver weight, hepatic blood flow, and antipyrine intrinsic clearance: Extrapolation of data to benzodiazepines and phenytoin. *J Pharmacokinet Biopharm* 1980; 8: 165–76.
- 30 Cheymol G. Effects of obesity on pharmacokinetics: Implications for drug therapy. *Clin Pharmacokinet* 2000; 39: 215–31.